

HOST-INDUCED MORPHOLOGICAL VARIATIONS IN THE
STRIGEOID TREMATODE *POSTHODIPLOSTOMUM MINIMUM*
(TREMATODA: DIPLOSTOMATIDAE). IV. ORGANS OF REPRODUCTION
(OVARY AND TESTES), VITELLINE GLAND, AND EGG¹

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ABSTRACT.— A variety of amphibian, reptilian, avian, and mammalian hosts were used in experimental development of *Posthodiplostomum minimum*. Results of this study indicate that the organs of reproduction (testes and ovary) as well as the vitelline gland and egg underwent host-induced morphological variations. Due to the lack of host specificity of *P. minimum* as well as the overlap of reported egg and body sizes of species of *Posthodiplostomum*, it is apparent that several reported species are not valid.

Throughout the literature, members of the trematode family Diplostomatidae have been classified and divided based upon the size and shape of the anterior and posterior testes, extent of vitelline gland, position of the ovary relative to the testes and the size of the egg (Dubois 1937, 1952, 1964, 1968, 1970). In the above cases, Dubois considered the strong host specificity supposedly demonstrated by strigeoids to be the basis for his extensive taxonomic revisions. In recent years several investigators have shown that strigeoid trematodes are not as physiologically host specific as previously suggested. Ulmer (1961) emphasized the need for additional experimental data relative to host specificity in order to assess the validity of Dubois's use of it as a major criterion for establishing taxonomic relationships. Palmieri (1973, 1975, 1976) clearly demonstrated that *Posthodiplostomum minimum* is able to develop to a gravid state in many host species within all vertebrate classes except fishes. It is doubtful, however, that host specificity in a strict sense is of value in differentiating species of *Posthodiplostomum*. Analysis of data presented in this paper clearly indicates that testes number, size and shape, ovary position, vitelline gland distribution and egg size undergo great morphological variations when experimen-

tally developed in amphibian, reptilian, avian, and mammalian hosts. Similar results dealing with morphological variation have been reported by Watertor (1967), Blankespoor (1971), Kinsella (1971), Campbell (1973), and Palmieri (1973, 1975).

MATERIAL AND METHODS

The material and method section of this paper has been reported by Palmieri (1976).

RESULTS AND DISCUSSIONS

OVARY.— Five variables were analyzed in studying the ovary of adult *P. minimum*, namely: (length, width, cross sectional area, ratio of length to width, and the variation in the position of the ovary relative to anterior and posterior testes). A complete analysis of the above data can be found in Table

1. Variations in ovary position of *P. minimum* are found in Figures 1-6 and Table 2.

The ovary of *P. minimum* commonly varies in position from anterior to the anterior testis (Figs. 1-2) to lateral (Fig. 3) or posterior (Figs. 4-6) to it. In worms recovered from all four classes of experimentally fed definitive hosts, the ovary was situated most commonly to the left of the anterior testis

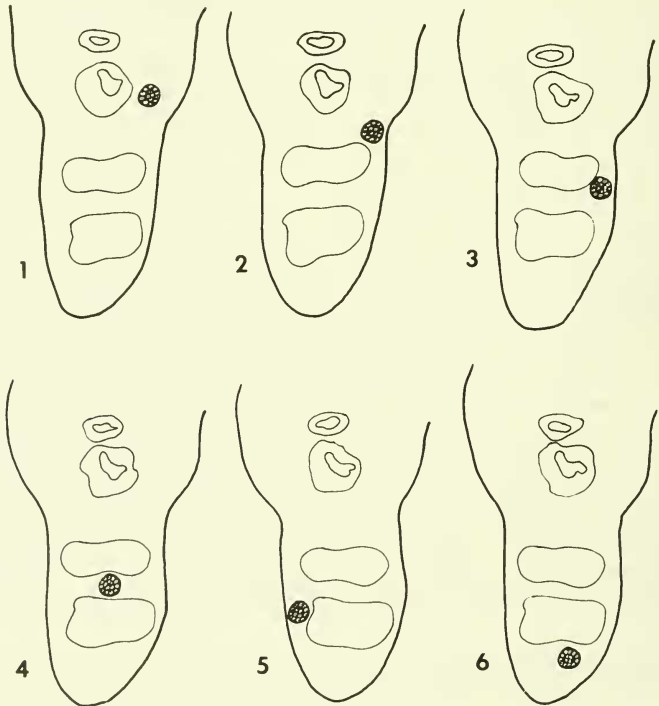
¹See Part II of this study, Great Basin Nat. 37:137, 1977.

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TABLE 1. Analysis of ovary measurements of *P. minimum* from experimental definitive hosts.*

Variable	Mean of Combined Host Class	Standard Deviation	Means			
			Amphibian	Reptilian	Aves	Mammalia
Ovary Length (OL)	0.027	0.007	0.029	0.013	0.031	0.030
Ovary Width (OW)	0.029	0.008	0.030	0.014	0.033	0.031
Cross-Sectional Area of Ovary (AOLXOW)	0.001	0.001	0.001	0.001	0.001	0.001
Ratio of Ovary Length to Width	0.882	0.241	0.930	0.542	0.940	0.976

*All measurements in mm.



Figs. 1-6. Variations in position of ovary as depicted in Table 2.

(Fig. 3). It was not uncommon to find the ovary in several of the above positions, however, within worms recovered from any given genus of cold-blooded definitive host. Variations such as these are much more common in poikilothermic than in homiothermic definitive hosts.

TESTES.—Ten variables were used in describing the size, number, and relationships of the anterior and posterior testes (anterior testes length and width, posterior testes

TABLE 2. Variation in ovary position relative to testes in adult *Posthodiplostomum minimum* recovered from experimental amphibian, reptilian, avian, and mammalian Hosts.

Experimental Host Class	Ovary Position Corresponding to Figures 1-6
Amphibia	2,3,5
Reptilia	2,3,6
Aves	1,2,3,4,5
Mammalia	2,3,4,5

TABLE 3. Analysis of testes measurements of *P. minimum* from experimental definitive hosts.*

Variable	Mean of Combined Host Class	Standard Deviation	Means			
			Amphibian	Reptilian	Aves	Mammalia
Anterior Testis Length (ATL)	0.048	0.017	0.042	0.031	0.066	0.057
Anterior Testis Width (ATW)	0.150	0.056	0.136	0.087	0.198	0.182
Posterior Testis Length (PTL)	0.050	0.017	0.048	0.027	0.068	0.053
Posterior Testis Width (PTW)	0.138	0.072	0.123	0.079	0.036	0.167
Cross-Sectional Area of Anterior Testis (AATLXPTL)	0.008	0.004	0.006	0.002	0.013	0.010
Cross-Sectional Area of Posterior Testis (APT LXPTW)	0.008	0.004	0.006	0.003	0.013	0.010
Ratio of Anterior Testis Length to Width (RATLPTW)	0.355	0.153	0.332	0.446	0.338	0.352
Ratio of Posterior Testis Length to Width (RPTLPTW)	0.408	0.399	0.527	0.251	0.371	0.308
Ratio of Cross-Sectional Area of Anterior Testis to Posterior Testis (RATXXPT)	1.016	0.658	1.174	0.522	1.067	0.989

* All measurements in mm.

length and width, cross-sectional area, ratio of testes length to width, ratio of the cross-sectional area of the testes, and testes number). An analysis of the above data can be found in Tables 3 and 4. A comparison of the morphological variation is drawn at the light level on Figures 9-21.²

The appearance of the testes, including their size, shape, and number, varied widely in specimens of *P. minimum* recovered from various vertebrate hosts. A definite trend is noticeable in accordance with the specific class of vertebrate host employed. In worms developed within avian hosts, anterior and posterior testes occupy a large area of the hindbody (Figs. 14, 16²) and are large, well

TABLE 4. Variations in testes number in adult *Posthodiplostomum minimum* recovered from experimental amphibian, reptilian, avian, and mammalian hosts.

Experimental Host Class	Testes Number
Amphibia	0,2
Reptilia	1,2
Aves	2
Mammalia	1,2

TABLE 5. Variation in vitelline gland distribution in adult *Posthodiplostomum minimum* recovered from experimental amphibian, reptilian, avian, and mammalian hosts.

Experimental Host Class	Vitelline Gland Distribution*
Amphibia	0,2,3
Reptilia	0,2,3
Aves	1,2
Mammalia	0,2,3

*0. No vitellaria present

1. From posterior region of the holdfast organ to region between acetabulum and oral sucker (corresponds to Fig. 13, Part III of this study).
2. From midregion of anterior testis to region between acetabulum and oral sucker (corresponds to Fig. 14, Part III of this study).
3. From midregion of holdfast organ to anterior region of acetabulum (corresponds to Fig. 15, Part III of this study).

developed, and approximately twice as wide as they are long. In mammalian hosts, worms have less normal-looking testes (Fig. 18²), but the latter are about three to four times as wide as long (Figs. 17, 21²), and are "dumbbell" shaped. Within poikilothermic hosts, testes of *P. minimum* appear even wider and sometimes show twists and diverticula (Figs. 11,13²). In several reptilian and mammalian hosts, no posterior testis appears. Several worms recovered from amphibian hosts showed no signs of testes development. Throughout all classes of hosts experimentally infected, worms from the more ecologically abnormal hosts tend to develop extremely wide testes with trends toward reduction in size of the posterior testis.

VITELLINE GLAND DISTRIBUTION.—Only the distribution of vitelline glands in the forebody and hindbody was considered in the adult *P. minimum* recovered during this investigation. This data was reported and diagrammed in Figures 13-15, Part III of this study.

In most specimens of *P. minimum* recovered from avian hosts, vitelline follicles are large and granular. Follicles are most heavily concentrated in an area extending from a region equidistant between acetabulum and oral sucker to the anterior half of the anterior testis. In some amphibian, reptilian, and mammalian hosts, vitelline distribution is identical to that found in the avian hosts, but follicles are smaller and less granular. In worms recovered from some reptilian and avian hosts (*Chrysemys*, *Columba*, and *Galus*), vitelline distribution is reduced to an area surrounding only the holdfast organ and the acetabulum. Worms recovered from amphibian hosts demonstrate a further reduction in vitelline distribution to a point where only a few follicles scattered around the holdfast are present. In some cases (amphibian, reptilian, and mammalian hosts) a complete absence of follicles resulted, even though age of the worms was sufficient to have permitted full development of these follicles.

²See Part II of this study, Great Basin Nat. 37:137, 1977.

EGG.—Six variables were analyzed in evaluating measurements of the eggs of *P. minimum*. Linear measurements consisted of egg length and width from worm specimens within a single host class. Two relationships of the above data were also studied, namely the ratio of the egg length means to the egg width means and the mean value for the cross-sectional area. A complete analysis of the above data can be found in Table 6.

Egg shape and development of *P. minimum* vary among experimental poikilothermic and homiothermic definitive hosts utilized in this investigation. *P. minimum* eggs from amphibian and reptilian hosts are small and round. Those recovered from amphibian hosts were not viable and no miracidial development could be detected. Egg size was largest from worms recovered from homiothermic hosts. The largest and most viable eggs of *P. minimum* are recovered from piscivorous avian and carnivorous mammalian hosts, and such eggs are characteristically oval and operculate. Egg number per worm varies from 1–2 in amphibian and reptilian to 1–5 (typically 3) in avian and mammalian hosts.

One of the principal factors contributing to the complex problems of speciation within the genus *Posthodiplostomum* and perhaps other genera of digenetic trematodes

has been the lack of experimental studies providing experimental data on host-induced variation at the intraspecific and interspecific levels. This study, based upon careful examination of over a thousand specimens of *Posthodiplostomum minimum* recovered from experimental hosts, emphasizes the pronounced morphological variation resulting from the rearing of *P. minimum* in different vertebrate definitive hosts, from any individual host class. Characters undergoing a significant change (F .01 level of significance or higher) include egg length and width, both ratio and area of the egg length means and the egg width means, and body length. These are the poorest characters for taxonomic identification of *P. minimum* since they show a significant morphological variation due to influences of the class of host in which development takes place.

This study provides evidence that the only significant morphological criteria for the determination of *Posthodiplostomum minimum* developed within any definitive host class are the ratio of the body length to body width, oral sucker cross-sectional area, acetabular index of length, acetabulum length, oral sucker width, and acetabulum width. Ratios between holdfast length and width and anterior testis length and width,

TABLE 6. Analysis of egg measurements of *P. minimum* from experimental definitive hosts.*

Variable	Mean of Combined Host Class	Standard Deviation	Means			
			Amphibian	Reptilian	Aves	Mammalia
Egg Length (EL)	0.078	0.007	0.034	0.025	0.084	0.085
Egg Width (EW)	0.030	0.054	0.028	0.020	0.058	0.057
Cross-Sectional Area of Egg (AELXEW)	0.004	0.005	0.013	0.001	0.005	0.004
Ratio of Egg Length to Width (RELEW)	1.445	0.095	1.324	1.266	1.444	1.481

*All measurements in mm.

as well as the oral sucker length and width, also aid in the taxonomic identification of this species.

It is apparent that, on the basis of those variables studied in this investigation, avian definitive hosts are by far the most suitable ones for *P. minimum*. Internal organs are

best developed with respect to size, and their position remains more constant than do those of worms reared in other vertebrate classes. Mammalian definitive hosts also serve as "normal hosts" to a lesser degree, for the extent of the vitellaria and the nature of the oral sucker are somewhat

TABLE 7. Comparison of body and egg measurements of reported species of *Posthodiplostomum* with those reported in this study.*

Species of <i>Posthodiplostomum</i>	Body Length		Body Width		Egg Length		Egg Width	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
<i>P. australe</i> (Dubois 1937)	0.766	1.150	—	—	0.080	0.091	0.052	0.062
<i>P. bi-ellipticum</i> (Dubois 1958)	1.190	1.230	0.530	0.560	0.078	0.078	0.056	0.056
<i>P. botauri</i> (Vidyarthi 1938)	1.200	1.562	0.256	0.720	0.080	0.080	0.064	0.064
<i>P. boydae</i> (Dubois 1969)	0.990	1.140	0.120	0.180	0.097	0.097	0.058	0.058
<i>P. brevicaudatum</i> (Nordmann 1832, Wisniewski 1958)	1.300	1.920	—	—	0.090	0.110	0.050	0.065
<i>P. cheni</i> (Yang 1959) Odening 1962	1.269	1.735	0.268	0.454	—	—	—	—
<i>P. cuticola</i> (V. Nordmann 1932, Dubois 1936)	0.670	2.310	0.220	0.920	0.073	0.110	0.051	0.088
<i>P. grande</i> (Diesing 1850) Dubois 1936	1.620	2.250	0.250	1.830	0.070	0.086	0.045	0.055
<i>P. grayi</i> (Verma 1936) Dubois 1938	0.830	1.077	0.210	0.390	0.070	0.093	0.042	0.074
<i>P. huesingi</i> Odening, 1962	1.080	2.600	0.300	1.600	0.073	0.110	0.051	0.080
<i>P. impraeputiatum</i> (Dubois 1934) Dubois 1936	1.200	2.110	0.160	0.430	0.092	0.103	0.055	0.065
<i>P. larai</i> (Refuerzo et Garcia 1937)	0.810	1.280	—	—	0.070	0.081	0.053	0.074
<i>P. macrocotyle</i> Dubois, 1937	0.890	1.200	0.200	0.290	0.077	0.089	0.050	0.057
<i>P. microsicya</i> Dubois, 1936	1.200	1.990	0.190	0.290	0.060	0.072	0.038	0.045
<i>P. milui</i> Fotedar et Reina 1965	—	—	—	—	—	—	—	—
<i>P. nanum</i> Dubois, 1937	0.410	0.950	0.090	0.610	0.062	0.076	0.042	0.048
<i>P. oblongum</i> Dubois, 1937	2.340	2.700	0.300	0.540	—	—	—	—
<i>P. opisthosilya</i> Dubois, 1969	0.910	1.160	—	—	0.092	0.096	0.057	0.070
<i>P. podicipitis</i> (Yamaguti 1939)	0.660	1.100	0.200	0.310	0.090	0.093	0.057	0.066
<i>P. prosostomum</i> (Dubois et Rausch) (Dubois 1952 comb. emend.)	2.300	2.530	0.450	0.500	0.082	0.090	0.052	0.058
<i>P. scardinii</i> (Shulman in Dubinin 1952)	—	—	—	—	—	—	—	—
<i>P. suni</i> N. T. Lee, 1964	1.840	1.890	0.301	0.301	0.073	0.073	0.053	0.053
<i>P. minimum</i> (MacCallum 1921)	0.840	1.750	0.160	0.600	0.073	0.091	0.048	0.064
This study	Mean		Mean		Mean		Mean	
<i>P. minimum</i> (Amphibia)	0.517		0.269		0.039		0.028	
<i>P. minimum</i> (Reptilia)	0.435		0.230		0.025		0.020	
<i>P. minimum</i> (Aves)	0.614		0.288		0.084		0.058	
<i>P. minimum</i> (Mammalia)	0.589		0.296		0.085		0.057	

*All measurements in mm.

more variable than in avian hosts. Poikilothermic hosts demonstrate marked variation and abnormal development. Especially among the Reptilia are extreme effects noticeable in developing worms, as evidenced by great reduction in body and organ size when compared with individuals reared in other host classes.

Throughout the literature, extensive synonymy exists for many species of *Posthodiplostomum*. Neither Dubois (1968, 1970) nor Yamaguti (1971) agree as to the number of valid species of this genus. As indicated by this investigation, few characters can be effectively utilized in separating adults of *P. minimum* reared in a great variety of poikilothermic and homiothermic hosts. Experimentally developed *P. minimum* from one or more classes of vertebrate hosts demonstrate such a wide degree of morphological variation that one doubts the validity of the number of species currently placed in the genus *Posthodiplostomum* as well as related genera in this strigeoid group. Certain characters such as body shape, ovary position relative to the testes, extent of vitellaria, relative size of fore- and hindbody, oral sucker shape, and host specificity have been and continue to be used in separating genera of the Diplostomini.

Even if one accepts *Posthodiplostomum* as a valid genus isolated morphologically and genetically from other related genera, the problem of speciation within the genus is even more confusing. Characters used by Dubois (1968, 1970) and Yamaguti (1971) (such as "testes tandem," "anterior testis oval," "posterior testis larger than the anterior testis") appear to be of little value, for this study has shown that host influences upon the adult *P. minimum* render such characters much too "plastic" to be of taxonomic importance. Because of inconsistencies in published accounts of egg sizes and descriptions of the 23 species of *Posthodiplostomum*, it is difficult to make a comparative analysis of them in relation to experimental results derived from this investigation. However, four variables (body length, body width, egg length, and egg width) have been compared (Table 7). Ex-

amination of data in this table makes it apparent that several reported species of *Posthodiplostomum* are probably not valid because of the considerable overlap in measurements of these structural features.

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